Technetium-99m labelled red blood cells scintigraphy and not iminodiacetic acid cholescintigraphy facilitates the discrimination of hepatic cirrhosis from fibrosis

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Abstract

Objective: This pilot study was designed to investigate the efficacy of technetium-99m labelled red blood cells (^{99m}Tc-RBC) compared with ^{99m}Tc-mebrofenin cholescintigraphy (^{99m}Tc-MHS), in the diagnosis of hepatic dysfunction at early stages. **Subjects and Methods:** Twenty four patients, 8 with hepatic fibrosis and 16 with cirrhosis, at Child-Pugh stage A to C and 20 age-matched controls were examined by ^{99m}Tc-RBC and by ^{99m}Tc-MHS. Dynamic acquisition and static images were semiquantitatively analused by studying the liver-to-heart (L/H) ratio estimated by both the ^{99m}Tc-RBC and ^{99m}Tc-MHS methods. The L/H ratios were compared between fibrosis, cirrhotic stages and controls, by Student's t test. Linear regression analysis of the L/H ratios for both methods has been applied in the whole study population. **Results:** Labelled RBC could statistically differentiate fibrotic from normal liver parenchyma (P<0.001), whereas the ^{99m}Tc-MHS could not (P: 0.13). The L/H ratios of cirrhotic lesions using both methods were significantly lower than those in controls: (P<0.000001 for ^{99m}Tc-RBC and P<0.0001 for ^{99m}Tc-MHS). Statistically significant difference was demonstrated by both modalities between fibrotic and cirrhotic lesions (^{99m}Tc-RBC: P: 0.003 and ^{99m}Tc-MHS: P: 0.024). **Conclusion:** Our study although in a limited number of patients suggested that as opposed to ^{99m}Tc-MHS, scintigraphic evaluation by ^{99m}Tc-RBC could be useful in the discrimination of patients with liver fibrosis, cirrhosis and in normal controls.

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Introduction

Fibrosis is prodromal to liver cirrhosis and accompanies cirrchosis. Fibrosis is due to increased synthesis of fibrous tissue and itself causes no symptoms. The scarring that accompanies fibrosis can lead to distorted blood flow through the liver and to early partial portal hypertension [1]. The evolution of fibrotic from cirrhotic liver tissue may have many clinical implications inline, such as bleeding or infection. Besides histology [2], imaging modalities have been used diagnostically in this aspect. Contrast-enhanced ultrasonography (CEUS), perfusion computed tomography (PCT), magnetic resonance imaging (MRI), transient elastometry (TE) and positron emission tomography (PET) have been used to differentiate early stages of liver cirrhosis [3, 4]. Furthermore, changes in hepatic microvasculature have been correlated [5]. Other researchers have studied liver circulation of blood by technetium-99m labelled red blood cells (^{99m}Tc-RBC), a well-established method in the detection of bleeding sites and hepatic hemangiomas [6-8]. In an original study we have previously reported that ^{99m}Tc-RBC could show impaired liver circulation of blood in early pre-cirrhotic stages where fibrosis predominates [8]. In this paper we have tried to compare and simplify this technique and additionally test the possible impact upon imaging on cholescintigraphy by mebrofenin cholescintigraphy (^{99m}Tc-MHS) of early stages of liver cirrhosis.

Patients and Methods

Inclusion criteria for the whole study population were based on fine needle aspiration biopsy (FNAB) at the 5th-6th intercostal space and on clinical assessment of the subjects studied. Regions of interest (ROI) drawings, were rectangular with standardized size in

each patient and were placed afar from the biopsy site at the left of the midclavicular line.

The present study protocol was in agreement with the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by our Institutional Review Committee. Informed consent in writing was obtained from each patient.

Twenty four patients (11 men and 13 women, mean age± SD: 56.8±10.2 years) were studied. Eight of them were at a fibrotic precirrhotic stage, 6 at a cirrhotic stage A, 5 at B, and 5 at stage C according to Child-Pugh classification [10]. Twenty age-matched controls were also submitted to ^{99m}Tc-RBC and ^{99m}Tc-MHS at consecutive sessions at 4 days intervals. Ultrasonography (US) was performed prior to scintigraphy in order to verify portal vein patency. All patients were fasting for at least 4h before undergoing the scinti-scan. In vivo labelled RBC by 740MBq Na^{99m}TcO$_4$ were intravenously (iv) as a bolus, 20min after the i.v. injection of stannous chloride in a dose of 0.03mL/kg. Cholescintigraphy was performed post the i.v. bolus of oxoethyl iminobisacetic acid-mebrofenin (^{99m}Tc-MHS) in a dose of 111-185 MBq. For both scintigraphic methods, immediately post-injection 10min dynamic acquisition study (for 2min at 2sec/frame and for 8min at 20sec/frame) was performed and followed by planar static images (1000kcounts) of the abdomen at 10min using a 20% energy window set at 140keV. In both studies (by RBC and MHS) image matrices of 128x128 were used in the dynamic studies and 256x256 in the static image. Equal ROI of standardized shape and size, were used over the left ventricle of the heart (H) and over the external part of the right lobe of the liver segments VII and/or VIII (L). Time-activity curves during 10min were acquired by both methods. Semiquantitative analysis for both RCB and MHS tests was performed by calculating the L/H ratio. Scores of L/H of all patients were calculated at 2min and 10min post injection, and evaluated statistically by Student's t test. Furthermore, the 10min L/H scores were compared by Student's t test with those of the controls. Linear regression analysis was performed for both methods between their L/H ratios of fibrotic and cirrhotic lesions of patients and controrols.

Results

In all cases, the L/H ratios did not differ significantly between the dynamic and planar static images.

The L/H ratio of labeled ^{99m}Tc-RBC was significantly lower in patients with fibrosis than in controls, P<0.001, whereas the L/H on the ^{99m}Tc-MHS study showed no real difference between patients with fibrosis and controls, P: 0.13 (Table 1). The L/H ratios in cirrhosis were significantly lower than those of controls for either ^{99m}Tc-RBC, (P<0.000001) and ^{99m}Tc-MHS, (P<0.0001).

The ^{99m}Tc-RBC and ^{99m}Tc-MHS L/H10min ratios between the different cirrhotic stages versus the controls, although the number of our patient group was small, produced the following indicative results: ^{99m}Tc-RBC in stage A vs controls: P<0.0002, in stage B vs controls: P<0.00001 (Figure 1), and in stage C vs controls: P=0.00007; ^{99m}Tc-MHS in stage A: P=0.006, in stage B: P=0.002, and in stage C: P=0.029 (Table 2).

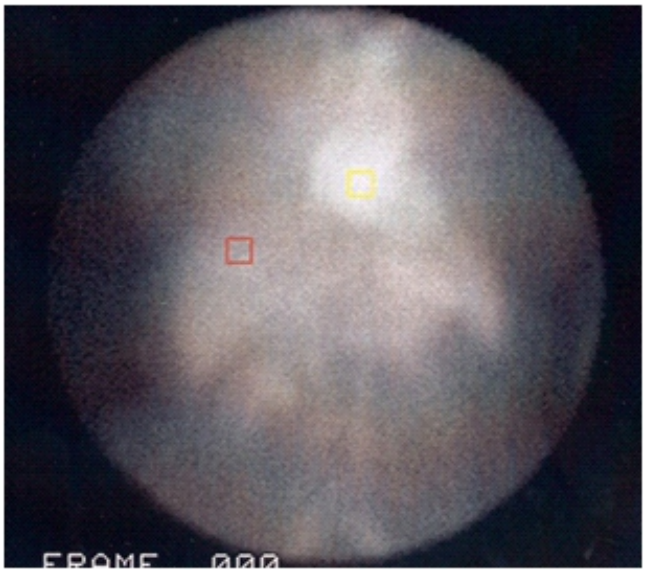

Figure1. Static image with ^{99m}Tc-RBC at 10min displaying hepatic fibrosis in a 43 years old patient; L/H ratio: 0.46.

Table 1. *Statistical correlations after comparing the ^{99m}Tc-RBC and ^{99m}Tc-MHS L/H ratios between patients with fibrosis or cirrhosis and controls*

Semi-quantitative Method	Cirrhosis	Fibrosis	Controls	Cirrhosis	Fibrosis	Cirrhosis
				vs Controls		vs Fibrosis
		Mean±SD		P values		
^{99m}Tc-RBC L/H	0.32±0.09	0.44±0.06	0.58±0.08	<0.000001	<0.001	0.003
^{99m}Tc-MHS L/H	5.06±3.6	9.73±2.8	11.9±3.1	<0.0001	0.13 NS	0.024

^{99m}Tc-RBC: ^{99m}Tc-labelled red blood cells; ^{99m}Tc-MHS: cholescintigraphy; L/H ratios: liver-to-heart ratios; NS: non-significant.

Table 2. *Statistical correlations after comparing the ^{99m}Tc-RBC and ^{99m}Tc-MHS L/H ratios between the different cirrhotic stages and controls*

	Cirrhosis (Mean±SD)			
	Stage A	Stage B	Stage C	Control group
^{99m}Tc-RBC L/H	0.38±0.06	0.3±0.037	0.16±0.02	0.58±0.08
	P<0.0002	P<0.00001	P: 0.00007	
^{99m}Tc-MHS L/H	8.25±1.78	2.58±2.04	1.3±0.02	11.9±3.1
	P: 0.006	P: 0.002	P: 0.029	

^{99m}Tc-RBC: ^{99m}Tc-labelled red blood cells; ^{99m}Tc-MHS: cholescintigraphy; L/H ratios: liver-to-heart ratios.

Statistically significant difference was demonstrated by both modalities between fibrotic and cirrhotic lesions (P: 0.003 and P: 0.024). Linear regression analysis regarding L/H ratios revealed significant coefficients of inverse correlation for the disease severity for both ^{99m}Tc-RBC and ^{99m}Tc-MHS, r: -0.835, P<0.001 and r: -0.814, P<0.001, respectively.

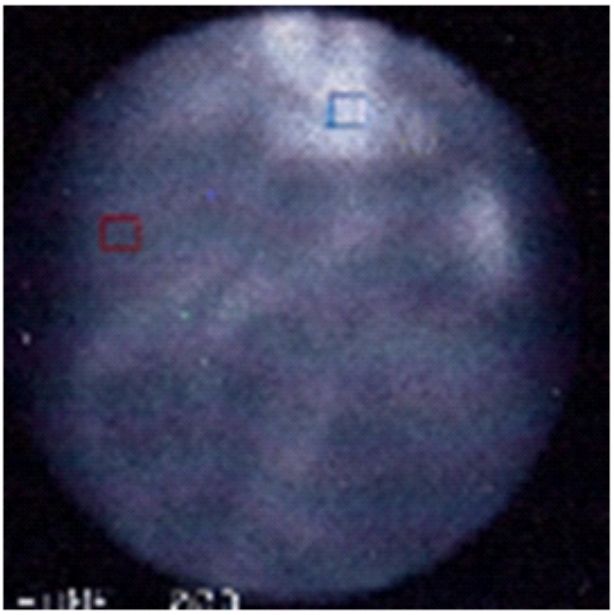

Figure 2. Static image with ^{99m}Tc-RBC at 10min, of a patient suffering from hepatic cirrhosis stage B; L/H: 0.28.

Discussion

The differential diagnosis between fibrosis and cirrhosis is very important because hepatic fibrosis can regress by appropriate treatment in its initial stages [12-14]. Results of this study showed a significant decrease of hepatic ^{99m}Tc-RBC pool in both fibrotic and cirrhotic lesions, being more prominent as liver disease progressed. This could be attributed to limited hepatic intravascular space to be followed by hemodynamic changes. Then activation of the hepatic perivascular stellate cells, specifically lipocytes or fat storing cells (Ito cells), initiates fibrosis. These and adjacent cells proliferate, becoming contractile cells called myofibroblasts, which produce in Disse spaces excessive amounts of abnormal matrix, consisting mainly of collagen types I, III, and IV, laminin, fibronectin, glycosaminoglycans, proteoglycans, elastin and form connective tissue membranes within the walls of the intralobular venous capillaries, and sinusoidal capillarization. Kupffer cells, injured hepatocytes, other cells and fibers, accummulate in the perisinusoidal spaces of Disse together with various factors, finally leading to liver fibrosis [13-16].

Many noninvasive imaging tests including CEUS, CT, MRI, TE and PET [2-5, 8, 9, 14-22] have detected cirrhosis and portal hypertension, splenomegaly and varices but they are not sensitive for the detection of advanced fibrosis, according to related references. Some imaging modalities such as chemical shift magnetic resonance [21], CEUS [19], and PCT [22] have been applied to assess the hepatic microvasculature, but despite being almost successful in distinguishing cirrhotic Child-Pugh stages [24], they have not been proved efficient in detecting hepatic fibrosis. Although fibrosis may appear as altered echogenicity on US or heterogeneity of signal on CT, these findings are nonspecific and may indicate only liver parenchymal fat. Modalities that assess liver stiffness like ultrasound elastography, MRI elastography, and acoustic radiation force impulse (ARFI) imaging may be useful but are not yet routinely used. Acoustic vibrations are applied to the abdomen with a probe and the measurement of a slow transmission through liver tissue indicates how stiff (i.e. fibrosed) the liver is but central obesity can decrease the diagnostic accuracy of all above tests, and also potentially limit their usefulness [13-15], as opposed to the ^{99m}Tc-RBC method. In our study, overweight patients were assessed. Overall the diagnostic accuracy of TE has been reported to be high for detecting cirrhosis, fair for sig-nificant liver fibrosis, and low for discriminating mild from significant fibrosis, which may limit the applicability of this technique in clinical practice [13-15].

By the use of PET and carbon-15 monoxide ($C^{15}O$) inhalation, researchers found a significantly greater semiquantitative mean tissue hepatic blood volume in normal controls as compared to chronic hepatitis or hepatic cirrhosis patients [9] but these methods are costly and not worldwide available for routine use.

An older quantitative measurement using carbon-11 monoxide-labelled RBC in PET studies described decrease of liver blood volume in patients with liver cirrhosis. The researchers suggested that this finding was due to hepatocyte swelling with fatty infiltration, fibrosis compression and narrowed sinusoids [17]. This method is also not widely available and difficult to apply in practice.

Liver FNAB remains the gold standard for diagnosing and staging hepatic fibrosis and for diagnosing the underlying liver disorder causing fibrosis. However, several draw-backs of liver biopsy, including its invasive nature resulting in a 10% to 20% risk of minor complications (e.g. postprocedural pain) and a 0.5% to 1% risk of serious complications (e.g. significant bleeding), its sampling error, and imperfect interobserver agreement in interpretation of histologic findings as well as its contraindications, warrant the need for an alternative diagnostic method. Other contraindications for FNAB include increased prothrombin time, international normalized ratio (INR) greater than 1.6, thrombocytopenia, platelet count less than 60.000, ascites, difficult body habitus, suspected hemangioma, suspected echinococcal infection and uncooperative patients [24].

Diagnostics and treatment of chronic diffuse hepatic diseases are some of the main problems in modern hepatology. Development of sequential stages of fibrosis culminating (means ending up) in cirrhosis and in liver cancer is the major pathway for progression of these diseases and this essentially determines unfavorable life-threatening prognosis and subsequent short time of survival of these patients [6, 20, 23, 24].

Hepatic blood flow changes as liver disease is deteriorating could actually explain decreased hepatic ^{99m}Tc-RBC radioactivity. Others have studied regional liver circulation and scintigraphic imaging of portal circulation with xenon-133 and found significantly and gradually decreased hepatic blood flow in patients with compensated (in terms of existing fibrosis) and decompensated cirrhosis as compared to patients with healthy liver, but this technique has never entered clinical practice, mainly due to the radioactivity burden of the radionuclide [18]. It is known that total blood volume is increased in patients with cirrhosis [25] and this could be a contradiction to our findings of decreased radioactivity emitted by the ^{99m}Tc-RBC in cirrhotic patients. The answer to this phenomenical mismatch is that the increase of total blood volume is attributed to plasma volume expansion rather than red blood cells mass [25].

The ^{99m}Tc-MHS modality determines the biliary tract patency [26] and has been proved useful in the diagnosis of interleukin-1 cholangiopathy [27]. In our study, ^{99m}Tc-MHS, which is excreted by the hepatocytes, sufficiently discriminated Child-Pugh B and C cirrhotic patients from healthy controls, but not the fibrotic or the Child-Pugh A cirrhotic patients [28].

The ^{99m}Tc-RBC test reflects hepatocyte damage through profound alterations in hepatic microcirculation, which appear even at early stages of liver disease with only mild hepatic fibrosis [5]. This may explain the better sensitivity of ^{99m}Tc-RBC versus ^{99m}Tc-MHS in the diagnosis of hepatic fibrosis.

Limitations of this study, apart from the limited number of patients studied, are that other liver malignant or benign diseases should also be examined by the above techniques.

In conclusion, our findings, although in a limited number of cases suggest that ^{99m}Tc-RBC scintiscan can be useful in differentiating liver fibrotic lesions from cirrhotic lesions and from normal liver parenchyma.

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The authors declare that they have no conflicts of interest.

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